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Novel mannitol based non-ionic surfactants from biocatalysis

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Abstract

Two new mannitol ester based non-ionic surfactants were synthesised using immobilised lipases. The synthesis started from mannitol derivatives having two of the six hydroxyls protected by an isopropylidene group. In the acylation step five different immobilised lipases were compared, for efficiency of substrate conversion. The commercial immobilised lipase from *Candida antarctica* B (Novozym 435) gave the highest conversion in the shortest time for both substrates. Selective hydrolysis of the isopropylidene group yielded 1-*O*-lauroyl-D-mannitol and 1,6-di-*O*-lauroyl-D-mannitol. Characterisation of the products was performed by ^{13}C NMR.

In the enzymatic step it has been thought that it is mainly the primary hydroxyl groups that are esterified. However, as detected from ^{13}C NMR, also other isomers were found. The formation of these isomers may be due to the non-specificity of the lipase, and to the acyl migration phenomenon.

Keywords: D-mannitol; Isopropylidene group; Non-ionic surfactant; Acylation; Immobilised lipase

1. Introduction

Carbohydrate fatty acid esters are non-ionic surfactants that can be obtained from renewable, cheap and easily available raw materials. They are used widely in the food, cosmetics and drug industries [1,2]. They have emulsifying properties, they are fully biodegradable, odourless, flavourless, non toxic and non skin irritant. Further they can be easily digested by the stomach as a carbohydrate-fatty acid mixture [3]. The hydrophilic-lipophilic balance (HLB) of these products can be modulated over a wide range by varying the chain length, the number of hydrocarbon chains of the carbohydrate molecule, or the degree of unsaturation of the chains [4]. Osipow et al. [5] reported the first paper describing an industrial process for carbohydrate ester production. This process depended upon a transesterification reaction between a carbohydrate and a fatty acid methyl ester. This takes place in the presence of a basic catalyst with DMF as a solvent.

At this time the industrial process for carbohydrate ester production is performed in anhydrous conditions at high temperatures and at reduced pressure. An alternative procedure makes use of concentrated sulphuric acid or sulphonic acids as catalysts, and high temperatures as well. These methods have the great disadvantages that accrue to high energy consumption, product coloration due to heat, and worst, the use of toxic solvents. In addition, industrial processes are not selective. They can furnish different product mixtures differing in the number of esterified hydroxyls or in the acylation position [6]. In the case of sugar alcohols, the process induces the formation of dehydrated products [7,8]. Although di- and tri-ester mixtures are convenient for many applications, chemical reactions that produce mono-esters selectively, in economical ways, open interesting perspectives in new applications. There is, therefore, strong motivation for the development of new and more selective synthesis methods and, also for economic purification procedures [9].

Enzymatic catalysis is able to overcome all the disadvantages of the chemical methods. The difficulty in the enzymatic synthesis of carbohydrate esters is due to the different polarity of the substrates (carbohydrate and fatty acid or its derivatives). This inhibits their dissolution in the same solvent. To overcome this problem two main strategies have been developed.

The first approach is the use of polar organic solvents or suitable mixtures. That procedure allows substrate dissolution while retaining enzymatic activity. Pyridine [10,11],

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THF [12] or a mixture of 2-methyl-2-butanol/DMSO (4/1) [13,14] have been used for this purpose.

The second approach is based on the polarity diminution of the carbohydrate, through the formation of an apolar derivative. The carbohydrate derivative is easily dissolved in an organic solvent together with the fatty acid (or its derivative). Alternatively, they can be melted and mixed in solvent-free conditions. As derivative agent, phenylboronic acid was used for its easy hydrolysis after the enzymatic step [15]. However, the use of this compound results in problems of biocompatibility on the final product.

In this work, the chemo-enzymatic syntheses of 1-*O*-lauroyl-D-mannitol, and 1,6-di-*O*-lauroyl-D-mannitol are reported by using a synthesis strategy previously proposed [7,16]. The mono- and di-fatty acid mannitol esters were synthesised from the reaction between a isopropylidene-D-mannitol derivative and vinyl laurate via enzymatic catalysis. This was then followed by selective chemical hydrolysis of the protecting groups. The enzymatic step (acylation) was catalysed by an immobilised lipase. In order to find the best catalyst, five different immobilised lipases were compared in terms of substrate conversion.

2. Experimental

2.1. Chemicals

Immobilised lipases (EC. 3.1.1.3) were gifts of Novo Nordisk—Bioindustriale s.r.l. (Milano, Italy); Novozym 435, lipase B from *Candida antarctica*; Lipozyme RMIM, lipase from *Rhizomucor miehei*. Powder lipases were purchased from Amano Enzyme Europe Ltd.; M Amano 10 was from *Mucor javanicus*, A Amano 6 was from *Aspergillus niger* and R Amano was from *Penicillium roqueforti*. The porous polypropylene powder MP1004 was a gift from Membrana GmbH Accurel Systems. Its characterisation for lipase immobilisation was reported in a previous work [17].

1,2-*O*-Isopropylidene-D-mannitol 97.0%, 3,4-*O*-isopropylidene-D-mannitol 97.0%, and $CHCl_3$ 99.9% were purchased from Aldrich. Vinyl laurate 99.0%, acetic acid 99.8%, 2-methyl-2-butanol 96.0% were purchased from Fluka. Hexane 99.0%, acetone 99.9% and DMSO 99.9% were purchased from Sigma.

2.2. Enzyme immobilisation

The enzymatic powders from Amano were immobilised by physical adsorption on a porous polypropylene powder called Accurel MP1004. The support (50 mg) was placed in 5 ml capped vials and wetted with 150 μl of 95.0% ethanol. The vials were filled with 4 ml of the lipase solutions in potassium phosphate buffer (20 mM and pH 6.0). All the vials were slowly rotated end-over-end overnight. The solutions were removed from each vial and checked for the lipase activity assay (*p*-nitrophenyl laurate), and protein assay to measure the residual activity and the residual protein content, respectively. The Accurel MP1004 particles were washed with fresh buffer until the residual water did not exhibit any enzymatic activity. The immobilised lipase preparations were dried overnight under vacuum.

2.3. Synthesis and purification of mannitol esters

A typical experiment was performed by preparing in anhydrous 2-methyl-2-butanol a solution 0.02 M in 1,2-*O*-isopropylidene-D-mannitol and 0.1 M in vinyl laurate. The reaction was started by adding 10 mg of immobilised lipase to 3 ml of the reagent solution. The reaction was carried out at 50 °C in an orbital shaker at 600 rpm in the presence of 100 mg of molecular sieves to keep the reaction medium dry. At the end of the reaction, the biocatalyst was removed by filtration and the solvent evaporated under reduced pressure.

Acid catalysed cleavage of the isopropylidene group was carried out using 10 ml of mixture of reaction with 10 ml of acetic acid (90.0%) at 80 °C, with magnetic stirring at 600 rpm for 3 h.

The reaction products were dissolved in $CHCl_3$ and then purified by column chromatography. A stationary phase of silica gel Merck 60 (230–400 mesh) and a solvent gradient chloroform: methanol from 10:1 to 1:1 v/v as mobile phase were used.

2.4. HPLC analysis

HPLC analysis was performed using a C-18 column (Merck) and monitored by an evaporative light scattering detector, Sedex 75 (Sedere, France). Analyses were carried out at 35 °C, at constant flow of 1 ml min^{-1} with an isocratic elution of CH_3CN:MeOH (1:1). Retention times of 2.47 min for 1,2-*O*-isopropylidene-D-mannitol; 4.01 min for 1-*O*-lauroyl-5,6-*O*-isopropylidene-D-mannitol; 2.92 min for 1-*O*-lauroyl-D-mannitol; 2.35 min for 3,4-*O*-isopropylidene-D-mannitol; 6.92 min for 1,6-di-*O*-lauroyl-3,4-*O*-isopropylidene-D-mannitol; 3.24 min for 1-*O*-lauroyl-3,4-*O*-isopropylidene-D-mannitol; 6.71 min for 1,6-*O*-di-*O*-lauroyl-D-mannitol; 2.29 min for D-mannitol; 3.23 min for lauric acid; 4.33 min for vinyl laurate.

2.5. ^{13}C NMR analysis

NMR measurements were performed using a Bruker Avance 300 MHz (7.05 T), equipped with a multinuclear probe operating at 75.475 MHz for the ^{13}C nucleus. The experiments were carried out at 25 °C using a standard variable-temperature control unity with an accuracy of ±0.5 °C. Appropriate amounts of sample (100 mg) were dissolved in DMSO-d_6 (2.5 ml) and such solutions were put inside NMR sample tubes with external diameter of 10 mm. The chemical shift scale refers to DMSO-d_6 solvent signals (39.52 ppm).

For the quantitative determination experimental conditions were chosen in such a way that the sum of the acquisition time and the delay time between two consecutive pulses (D1) was more than five times the spin-lattice relaxation time (T_1). ^{1}H-decoupled ^{13}C NMR spectra were recorded exploiting an inverse gated pulse sequence to suppress the nuclear overhauser effect (nOe) and using a 90°-pulse (11.50 μs). Spectral data were treated using Bruker WINNMR software. Deconvolution and curve fit were applied to obtain the area of each peak.

The spin-lattice relaxation times were measured via the classical inversion recovery sequence (180-τ-90-D1) by acquiring the partially relaxed spectra at 14–18 different τ values.

When required, in order to discriminate the various carbohydrate carbons, a composite pulse sequence, ^{13}C DEPT-135, which yields spectra with positive CH and CH_3 signals and negative CH_2 signals, was used.

All analyses were carried out at least in triplicate. Errors, never larger than 3%, were found.

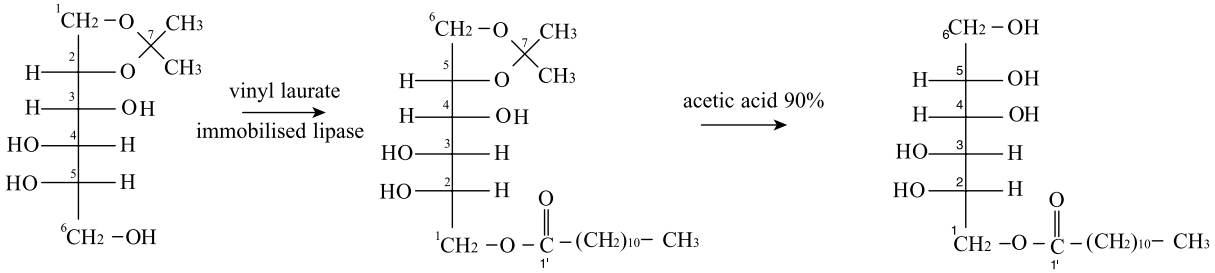
3. Results and discussion

The path of the syntheses of the mannitol esters was carried out in accordance with the following procedure. An isopropylidene-D-mannitol derivative, dissolved in anhydrous 2-methyl-2-butanol and in the presence of an excess of vinyl laurate, was acylated by using an immobilised lipase. The successive step was the chemical, selective, hydrolysis of the isopropylidene protecting group. The steps for the syntheses of 1-*O*-lauroyl-D-mannitol and 1,6-*O*-di-*O*-lauroyl-D-mannitol are reported in Scheme 1 and Scheme 2, respectively.

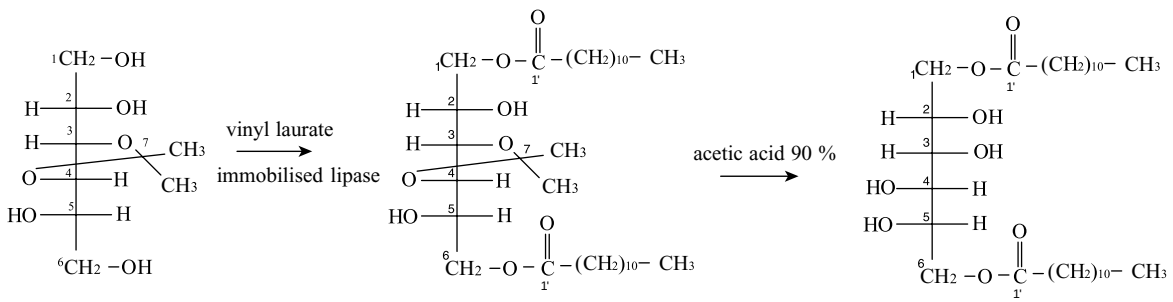
3.1. Screening of the immobilised lipases

Five immobilised lipases were compared in acylation efficiency of the isopropylidene-D-mannitol in organic solvent. Two of them were immobilised commercial lipase preparations, Novozym 435 and Lipozyme RMIM, that have been extensively used in the biocatalysis research area [18–20]. The other three were powder preparations immobilised by adsorption, in our laboratory, on Accurel MP1004 a polypropylene powder. As recently shown, this support is mainly macroporous, but contains also a relevant percentage of mesopores that are enough large to allows lipases adsorption [17].

Fig. 1 shows the conversion of 1,2-*O*-isopropylidene-D-mannitol into 1-*O*-lauoryl-5,6-*O*-isopropylidene-D-mannitol catalysed by five different immobilised lipases. Novozym 435 (*C. antarctica* B) reached 100% of conversion after 24 h. The lipozyme RMIM (*R. miehei*) reached 95% of conversion after 74 h. The other immobilised lipases from *M. javanicus*, *A. niger* and *P. roqueforti*—achieved, after 72 h, 38, 22 and 18% in conversion, respectively. For this reaction Novozym 435 and lipozyme RMIM show optimal behaviour. In the case of the first lipase the reaction must be stopped at 24 h, because after this time the product hydrolysis occurs. The other immobilised lipases give too low values of conversion in the time range investigated.



Scheme 1. Synthesis of 1-*O*-lauroyl-D-mannitol and C atom numeration of reagent and products.



Scheme 2. Synthesis of 1,6-di-*O*-lauroyl-D-mannitol and C atom numeration of reagent and products.

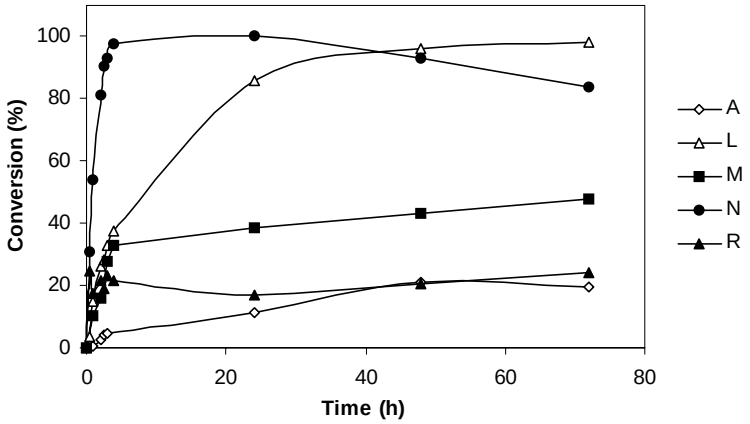


Fig. 1. Conversion of 1,2-*O*-isopropylidene-D-mannitol catalysed by the immobilised lipases: *Aspergillus niger* (A); *Rhizomucor miehei* (L); *Mucor javanicus* (M); *Candida antarctica* B (N) and *Penicillium roqueforti* (R). All reactions were carried out in 2-methyl-2-butanol at 50 °C.

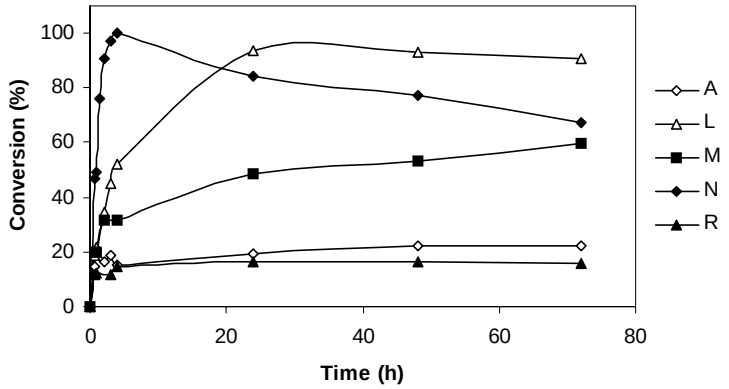


Fig. 2. Conversion of 3,4-*O*-isopropylidene-D-mannitol catalysed by the immobilised lipases: *Aspergillus niger* (A); *Rhizomucor miehei* (L); *Mucor javanicus* (M); *Candida antarctica* B (N) and *Penicillium roqueforti* (R). All reactions were carried out in 2-methyl-2-butanol at 50 °C.

Fig. 2 shows the conversion of 3,4-*O*-isopropylidene-D-mannitol to form the acylated compound, catalysed from the same immobilised lipases as before. For *C. antarctica* B lipase, two different compounds are formed. These are the mono- and the di-acyl-isopropylidene mannitol. Not all the enzymes were able to produce the di-acyl product over the range of time explored. But some of them stopped at the mono-acyl product or gave a mixture of both. Table 1 shows the yields of mono- and di-acyl-isopropylidene-D-mannitol obtained at the highest conversion of reagent. Again in this case, the Novozym 435 was the most active enzyme achieving 100% of conversion after only 4 h. The reaction gave a yield 37.3% of the mono-acyl-isopropylidene-D-mannitol and 62.7% of di-acyl-isopropylidene-D-mannitol. After this time, as in the previous case, the lipase catalysed the product hydrolysis giving a decrease of the conversion.

Table 1
Yields of 1-*O*-lauroyl-3,4-*O*-isopropylidene-D-mannitol (LIM); 1,6-di-*O*-lauroyl-3,4-*O*-isopropylidene-D-mannitol (DLIM) at the highest conversion value reached for the five immobilised enzymes: *Candida antarctica* B (N), *Rhizomucor miehei* (L), *Mucor javanicus* (M), *Penicillium roqueforti* (R) and *Aspergillus niger* (A)

	Lipase				
	N	L	M	R	A
Time (h)	4	24	336	48	48
Conversion (mol%)	100	93.7	74.8	16.6	22.3
$Yield_{LIM}$ (mol%)	37.3	81.7	69.5	16.6	22.3
$Yield_{DLIM}$ (mol%)	62.7	12.0	5.3	0	0

The Lipozyme RMIM reached its highest value of conversion (94%) after 24 h. After this time the conversion decreased slightly. In this case the most abundant compound was the mono-acyl-isopropylidene-D-mannitol with a yield of 82%, and a yield of 12% in di-acyl-isopropylidene-D-mannitol.

The immobilised lipase M was less active than the other two enzymes. Indeed, it reached its highest conversion value (74.8%) after 14 days. Even in this case, the most abundant compound was the mono-acyl-isopropylidene-D-mannitol with a yield of 69.5% versus a yield of 5.3% in di-acyl-isopropylidene-D-mannitol.

The last two immobilised lipases, R and A, behaved in a similar way. They reached after 48 h a maximum conversion of 16.6 and 22.3%, respectively. In both cases only the mono-acyl compound was obtained.

As already observed, among all the immobilised enzymes tested, the *C. antarctica* lipase B shows a singular behavior. Once the maximum of conversion is reached, the product hydrolysis occurs. This phenomenon must be somehow related to the enzyme or its support. The support is a macroporous acrylic resin for the *C. antarctica* lipase B, a macroporous anion exchange resin for the *R. miehei* and a macroporous polypropylene powder for the other three enzymes. The different supports could adsorb the product near the active site of the lipase. This phenomenon may affect differently the equilibrium and is likely to favour the removal of the acyl group from the mannitol backbone in the case of the Novozym 435. At this stage, however a deep investigation on this point is behind the aim of this work.

3.2. Key factors of the enzymatic acylation

In order to maximise the yield in the enzymatic step, the following key factors were found to be important.

- Absolute absence of water in the reaction medium. Water influences the enzymatic activity in organic media [21–23]. Usually each enzyme shows an optimum value of water activity that is independent of the different solvent used [24]. In our reactions a very low content of water was sufficient to produce lauric acid from the concurrent reaction of vinyl laurate hydrolysis. For this reason, it was essential to keep both the reagent solutions and the enzyme preparations dry before and during the reaction by using molecular sieves.
- The vinyl laurate is used as acylation agent, instead of another ester, because during the enzymatic reaction vinyl alcohol is formed. This compound transforms to its more stable tautomer, the ethanal, so driving the equilibrium towards the products.

3.3. Hydrolysis of the protecting groups

Having determined that the best catalyst for the acylation of the two protected sugar alcohols is the immobilised *C. antarctica* lipase B, the compounds obtained were submitted to the required sequential step: the selective chemical removal of the isopropylidene groups. This reaction was carried out by suspending the products of the previous reaction in acetic acid 90% at 80 °C for 3 h. The yields in the final products, the non-ionic surfactants 1-*O*-lauroyl-D-mannitol and 1,6-di-*O*-lauroyl-D-mannitol, were higher than 95% in both cases as determined by HPLC analysis.

3.4. ^{13}C NMR analysis

Through ^{13}C NMR spectroscopy the various steps of the syntheses were followed straightforwardly, so allowing the correct determination of the main, along with the secondary (hydrolysis), reaction products.

As an example, Fig. 3 shows the ^{1}H-decoupled ^{13}C NMR spectrum of the 1,6-di-*O*-lauroyl-D-mannitol synthesis reaction products before hydrolysis of the protecting group, and after a purification step through column chromatography. The occurrence in the sample of two different molecules is evident from the analysis of the NMR signals in the spectral regions of the isopropylidene group quaternary carbon, of the carbohydrate carbons moiety and, finally, of the carboxylic carbons. The highest signals clearly belong to 1,6-di-*O*-lauroyl-3,4-*O*-isopropylidene-D-mannitol while the lowest can reasonably be attributed to 1,5-di-*O*-lauroyl-

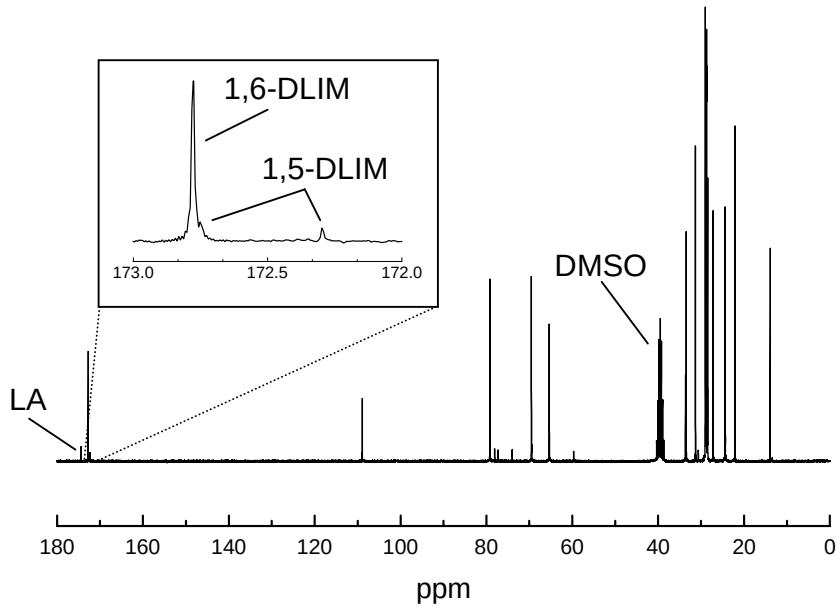


Fig. 3. ^{1}H-decoupled ^{13}C NMR spectrum of reaction products of 1,6-di-*O*-lauroyl-3,4-*O*-isopropylidene-D-mannitol (1,6-DLIM) synthesis. The signals of the isomer 1,5-di-*O*-lauroyl-3,4-*O*-isopropylidene-D-mannitol (1,5-DLIM), of the by-product lauric acid (LA), and of the solvent (DMSO) are indicated.

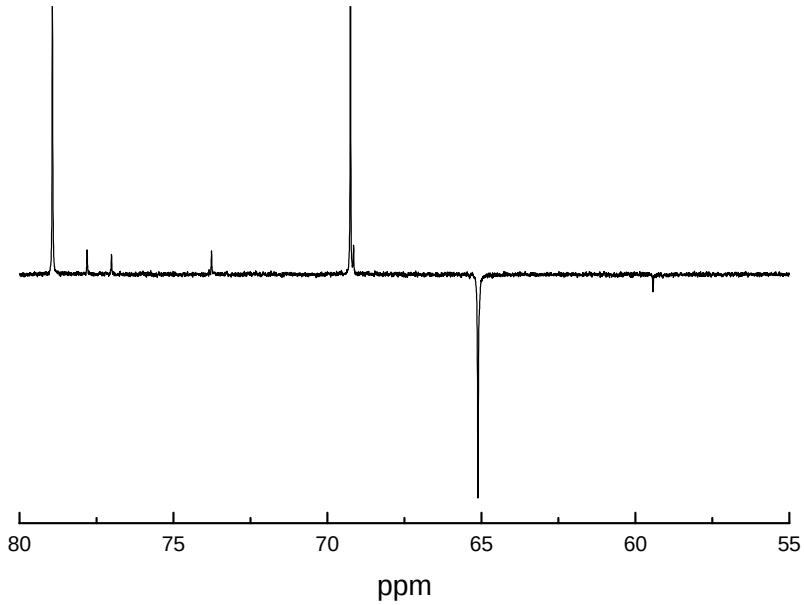


Fig. 4. ^{1}H-decoupled ^{13}C DEPT—135 NMR spectrum of the carbohydrate carbons region of 1,6-di-*O*-lauroyl-3,4-*O*-isopropylidene-D-mannitol.

3,4-*O*-isopropylidene-D-mannitol. These assignments are reinforced by information available from the spectra of Fig. 3 and Fig. 4. Here the ^{13}C DEPT-135 (see experimental) NMR spectrum of the same sample, expanded in the region of the carbohydrate carbons, is shown. As can be observed in the inset of Fig. 3, three NMR signals that belong to ester carboxylic carbons were detected. Moreover, as determined from a quantitative analysis, the lowest signals possess the same area. These facts fit neatly with the idea of a non-symmetric di-ester molecule. Further, in Fig. 4, five (instead of six) small signals can be distinguished. Therefore, one signal from the 1,6-di-*O*-lauroyl-3,4-*O*-isopropylidene-D-mannitol molecule overlaps one signal from the other molecule. Indeed, the sole CH_2 signal with low intensity that can be detected (at 59.70 ppm) originates from the CH_2-OH group of an asymmetric molecule, and it is clear that the signal at 65.38 ppm overlaps the other CH_2 signal from the same molecule. It is also worth noticing that a lauroyl residue linked in position 5 is unlikely to produce a resolvable chemical shift alteration in position 1.

These evidences more than adequately support the hypotheses concerning the 1,5-di-*O*-lauroyl-3,4-*O*-isopropylidene-D-mannitol molecule formation.

Similar considerations allowed the characterisation of the 1-*O*-lauroyl-5,6-*O*-isopropylidene-D-mannitol.

An accurate quantitative analysis via NMR was also executed. Reaction products of the two syntheses were found to be mixtures composed of, respectively, 88% 1,6-di-*O*-lauroyl-3,4-*O*-isopropylidene-D-mannitol and 12% 1,5-di-*O*-lauroyl-3,4-*O*-isopropylidene-D-mannitol and 65% 1-*O*-lauroyl-5,6-*O*-isopropylidene-D-mannitol and 35% of other isomers (results are expressed in terms of moles percent). The high percentage of by-products detected in the 1-*O*-lauroyl-5,6-*O*-isopropylidene-D-mannitol synthesis deserves particularly to be noted.

NMR carbons assignments of the species recognised are reported in Table 2.

3.5. Considerations on the formation of isomers in the acylation step

When the *C. antarctica* lipase B was systematically used, besides the main product which are the esters of the primary hydroxyls, some other isomers were also obtained. The detection of these isomers was accomplished via ^{13}C NMR techniques. HPLC was not able to distinguish these compounds. Two different causes may be responsible for the formation of the isomers, e.g. the acyl migration, and the non-specificity of the *C. antarctica* lipase B towards the primary hydroxyls of the D-mannitol. The presence of these isomers was higher in the case of 1-*O*-lauroyl-5,6-*O*-isopropylidene-D-mannitol than for 1,6-di-*O*-lauroyl-3,4-*O*-isopropylidene-D-mannitol (35 mol.% versus 12 mol.%).

Let us consider the first cause: the acyl migration. Mannitol backbone is similar to glycerol, because of the occurrence of vicinal hydroxyls. Hence the mannitol esters are expected to show a behaviour similar to that found for mono- or diglycerides [25,26]. It is well known that these two classes of compounds undergo acyl migration between 1- and 2-positions of glycerol through an intramolecular mechanism [18]. This phenomenon occurs for thermodynamic reasons [27,28]. In our compounds acyl migration gives the 1,5-di-*O*-lauroyl-3,4-*O*-isopropylidene-D-mannitol from the 1,6-di-*O*-lauroyl-3,4-*O*-isopropylidene-D-mannitol, and similarly other some isomers of the 1-*O*-lauroyl-5,6-*O*-isopropylidene-D-mannitol.

Table 2
^{13}C NMR in DMSO-d_6

	δ(**1**) (ppm)	δ(**2**) (ppm)	δ(**3**) (ppm)	δ(**4**) (ppm)	δ(**5**) (ppm)	δ(**6**) (ppm)	δ(**7**) (ppm)	δ(**8**) (ppm)
C1	66.82	66.69	66.91	62.99	65.38	66.82	–	–
C2	75.14	68.04	68.31	72.88	69.52	69.09	–	–
C3	70.10	69.76	69.31	79.07	79.20	68.26	–	–
C4	70.06	69.90	69.48	–	–	–	–	–
C5	70.89	75.06	71.20	–	–	–	–	–
C6	63.72	66.81	63.85	–	–	–	–	–
C7	107.92	107.99	–	108.28	109.07	–	–	–
C1′	–	173.03	173.10	–	172.79	173.15	170.30	174.36
C2′[a]	–	–	–	–	–	–	141.20	–
C3′[a]	–	–	–	–	–	–	97.81	–

Chemical shifts of C atoms according to the numeration in Scheme 1 and Scheme 2: 1,2-*O*-isopropylidene-D-mannitol (**1**); 1-*O*-lauroyl-5,6-*O*-isopropylidene-D-mannitol (**2**); 1-*O*-lauroyl-D-mannitol (**3**); 3,4-*O*-isopropylidene-D-mannitol (**4**); 1,6-di-*O*-lauroyl-3,4-*O*-isopropylidene-D-mannitol (**5**); 1,6-di-*O*-lauroyl-D-mannitol (**6**); vinyl laurate (**7**) and lauric acid (**8**).

[a] These signals are due to the carbons of the double bond of the vinyl group in (**7**)

Regarding the second cause, is important to remind that the *C. antarctica* lipase B shows a very different behavior depending on the substrate. When a triacylglyceride is involved in a hydrolysis or in a transesterification reaction, the *C. antarctica* lipase B acts indifferently on the three positions of the glycerol backbone. In the case of a glucopyranoside acylation it was found that the *C. antarctica* lipase B showed a very high regio-specificity towards the primary hydroxyl of the sugar [29]. Here because of mannitol structure, the *C. antarctica* lipase B is expected to behave similarly to the case of glycerol, e.g. no specificity towards primary hydroxyls. By considering the two esters of the isopropylidene-D-mannitol, it is very likely that secondary hydroxyls are acylated in the case of 1,2-*O*-isopropylidene-D-mannitol to give the different isomers. On the contrary in the case of 3,4-*O*-isopropylidene-D-mannitol the occurrence of the isopropylidene group prevents the acylation of the 2 and 5 positions for sterical reasons.

The different combination of these two phenomena accounts for the higher percentage of isomers of the 1-*O*-lauroyl-5,6-*O*-isopropylidene-D-mannitol compared to those of the 1,6-di-*O*-lauroyl-3,4-*O*-isopropylidene-D-mannitol.

The encouraging results here obtained through biocatalysis may open new possibilities for widen applications of biocompatible carbohydrate esters.

4. Conclusions

Two new mannitol-based surfactants were obtained by following a synthesis strategy via acylation of two different isopropylidene-D-mannitol groups. As protecting group, acetone is less expensive than other protecting agents as phenylboronic acid. It is also more biocompatible. The *C. antarctica* lipase B gave the highest conversion in the lower time for both substrates. In the reaction with 3,4-*O*-isopropylidene-D-mannitol this enzyme gave also the highest percentage of diester compared to the other lipases. Besides the main products, obtained from the acylation of primary hydroxyls, different isomers were also obtained as by-products. The acyl migration and the unspecificity of the lipase are the most likely reasons of this fact.

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